File S1

ADDITIONAL METHODS

Construction of the vectors: The vectors were constructed by amplifying the portions of *ie1*, *lef1*, *lef3* and *p74* genes encoding the *immediate early 1* gene, late expression factors 1, 3 and envelope protein of BmNPV, respectively, using primers carrying specific restriction enzyme sites. Similarly, *gfp* gene of pPIGA3GFP (Kanginakudru *et al.* 2007) was amplified. The primers used for the amplification of the gens are listed in the TABLE S1**.** The amplicons were cloned into the T-overhangs of pCRII TOPO vector (Invitrogen) (FIGURE S1). The resultant plasmids were labeled as Topo-*ie1*, Topo-*lef1*, Topo-*lef3*, Topo-*p74* and Topo-*gfp.* The targeted regions of *ie1*, *lef1*, *lef3*, and *p74* are as mentioned in File S2. These vectors having right inserts were confirmed by restriction digestions and DNA sequencing. These baculoviral genes produce virus specific proteins and do not share any homology to known insect or human genes.

The pTopo clones were transformed into one shot INV110 chemically competent *E. coli* cells (Invitrogen). Blue/White screening was performed on LB agar plates containing 40 µl of X-Gal (40 mg/ml) and 40 µl of isopropyl β-D-thiogalactoside (IPTG, 100 mM). White colonies were picked up for insert analysis through digestion. Colonies that gave right inserts of expected size were picked up. For DNA sequencing, 250 ng of plasmid was used in a sequencing reaction that contained 8 µl of Ready reaction mix (BigDye terminator, BDTv 3.0, Applied Biosystems, Foster City, CA) and 5 picomoles of M13 sequencing primers. The cycling conditions used were as follows: 25 cycles of 96°C for 10 sec, 50°C 5 sec, 60°C 4 min. Samples were ethanol precipitated, washed with 70% ethanol and resuspended in Hi-Di^{TM}formamide (Applied Biosystems). The sequencing was carried out in ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and the intactness of each gene fragment was confirmed. The constructs were obtained in a series of cloning steps as described below:

1. A 316 bp fragment of *lef-3* was cloned into pA3DS-FSG vector backbone (7624 bp) using *Sal*I-*Nhe*I sites to generate single gene construct, pA3ΔS-lef3 (6057 bp). The transformants were verified by *Xmn*I (an internal site of *lef3*) restriction digestion and *Xmn*I/*Sca*I double digestion. *Xmn*I digestion linearizes to 6.05 kb fragment of 1-gene construct and double digestion with *Sca*I (a site in backbone) released 323 bp product or insert size and 5.73 kb fragment as per DNA strider map pattern.
2. A 310 bp fragment of *ie-1* was cloned in to pA3ΔS-lef3 vector using *Bsp*EI-*Sal*I sites to generate pA3ΔS-ie1lef3SG, a two gene construct (5929 bp). The transformants were checked upon sequential digestion using *Xmn*I*/Hpa*I restriction system. *Xmn*I linearization of 2-gene construct gave rise to 5929 bp fragment and *Xmn*I/*Hpa*I sequential digestion gave rise to expected three bands of 339 bp, 1071 bp and 4519 bp fragments.

3. An 800 bp fragment of *gfp* was cloned into pA3ΔS-ie1lef3 vector backbone using *Pvu*I-*Bam*HI sites to generate pA3ΔS-gfpie1lef3, a three gene construct (6665 bp). The transformants were checked upon *Pvu*I/*Eco*RI restriction analysis which gave rise to 834 bp insert and 5834 bp backbone.

4. A 326 bp *lef-1* clone was added to pA3ΔS-gfpie1lef3 vector backbone using *Bsp*EI/*Bam*HI to generate pA3ΔS-gfplef1ie1lef3 (6650 bp) a four gene product. The transformants were confirmed through *Hpa*I and *Nde*I restriction digestions which gave rise to 3 fragments of expected lengths 188 bp, 1410 bp, 5040 bp and 2 fragments of expected length 1361 bp and 5289 bp, respectively.

5. A 310 bp fragment of *p74* was cloned in to pA3ΔS-gfplef1ie1lef3 vector backbone using *Age*I-*Xba*I sites to generate a five gene construct, pA3ΔS-gfple1ie1lef3p74 (6160 bp). The transformants were confirmed through *Sal*I restriction digestion. It gave rise to 520 bp insert and 5640 bp backbone.

The five gene sense oriented construct, pA3ΔS-gfplef1ie1lef3p74 was digested with internal *Nhe*I-*Xba*I sites to generate sense and antisense oriented pool of fragment of five genes. Transformants were screened for antisense oriented plasmids. This was confirmed by *HindIII/XmnI* digestion, wherein the antisense colony gave rise to 1.3 kb fragment whereas sense oriented colony gave rise to 2.2 kb fragment.

Sense construct - The *A3:gfp* fused promoter along with four genes and poly A tail were cloned into *pPiggyP25il2-(3XP3-GFP)TQ* using *BglII-NheI* and *BglII-XbaI* sites to generate *pPiggyMG(+)3XP3-GFP* vector (FIGURE 1).

Antisense construct - The antisense fragment obtained as mentioned above was cloned into *pPiggyP25il2-(3XP3-DsRed2)TQ* to generate *pPiggyMG(-)3XP3-DsRed2* vector (FIGURE 1).

6. Inverted Repeat Construct - The above *A3:gfp* fused promoter along with four genes was cloned into pP25(sp*)DsRed vector backbone using *Bgl*II-*Age*I sites to generate sense fragment with spacer region, an intermediary vector form pP25(sp*)DsRed-A3ΔS Sense.Spacer.

7. The antisense fragment of five genes was cloned into intermediary form of sense-spacer vector backbone using *Sse*8337I-*Afl*II sites to generate flip-flop form of multigene construct, pP25(sp*)DsRed-A3ΔS Sense.Spacer.Antisense.Poly A region.

8. The intermediary shuttle vector, pP25(sp*)Sense-Spacer-Antisense vector was digested with *BglII* and *SfiI* sites to generate inverted repeat. This insert was cloned into *PiggyP25IL2-(3XP3-DsRed2)TQ* using *BglII/SfiI* sites to generate *pPiggyA3gfplef1ie1lef3p74.spacer.p74lef3ie1lef1gfp.3XP3-DsRed2 (pPiggyMG(+/-)3XP3-DsRed2)* vector with *DsRed2* as the selection marker gene (FIGURE 1).

Polymerase Chain Reaction: A typical PCR reaction consisted of 20 µl final volume with 5 pmol each of forward and reverse primers. The PCR amplification was performed in 10 mM Tris-HCl, (pH 8.3 containing 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin and 0.01% Triton X-100), 1 mM dNTPs and 0.5 U of *Taq* DNA Polymerase (NEB, UK) per reaction. Thermal cycling was carried out in a thermal cycler (PE9700, Applied Biosystems) using the following conditions: initial denaturation of 3 min at 95°C; 35 cycles of 30 sec at 94°C; 30 s at 52°C (as per the *Tm* of the respective gene) and 2 min at 72°C, and final extension of 10 min at 72°C. The PCR products were quantified on agarose gel, purified using Qiagen PCR purification columns, according to the manufacturer's protocol.